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BLOCKING OF PRODUCTION AND ACTIVITY OF INTERFERON BY AN INHIBITOR INDUCED BY VACCINIA VIRUS

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T. A. Bektemirov
and
A. Ye. Gummenik

We have established earlier(1) that the strain of vaccinia virus which had been used at the Moscow Institute of Virus Preparations for the preparation of vaccine does not induce the formation of interferon in the cultures of chicken fibroblasts. However, after having been inactivated at 56° in the course of 50 minutes, it clearly shows this ability. The production of interferon also does not occur when active and inactivated viruses of vaccinia virus are introduced into the culture at the same time. A similar phenomenon was described by Wagner and Huang(6), who observed the inhibition of the production of interferon by the cells infected by the virus of Newcastle disease when they were under the effect of vesicular stomatitis virus. Hermodsson(4), Vilcek and Stancek(5) also established the ability of some viruses to inhibit the production and effect of interferon. There appeared a hypothesis that the above-mentioned viruses induce the production of an inhibitor which blocks the ability of the cells to produce interferon. Yu. Z. Gendon(3), who had undertaken a study in this direction, discovered an inhibitor which was blocking not the formation but the effect of interferon.

The present paper reports on the production by the cells of chicken fibroblasts of an inhibitor of nonviral nature blocking the formation and effect of interferon.

Materials and Methods

We used the vaccinia and Chikungunya viruses which were described by us earlier[1,2]. The method of obtaining and titrating interferon was described in the same reports.

In the experiments on the inhibitor induction we used a modified method by Yu. Z. Gendon[3].

Forty-eight hours after their inoculation into the Roux matrasses, chicken fibroblasts were infected with the vaccinia virus at a multiplicity of 2-3 TCD_{50} (tissue cytopathogenic dose) per 1 cell and were placed in a thermostatically-controlled chamber at 37°. One hour later the cells were washed three times with Hanks' solution, we introduced 100 ml of medium No. 199 into each matrass and placed them in a thermostatically-controlled chamber for two hours. Then the medium was removed and the cells were mechanically removed from the surface of the glass and suspended in 15 ml of medium No. 199. The contents of three matrasses were put together and the cell suspension was exposed to ultrasonic vibration for 10 minutes (frequency 800 kilohertz, power 10 watt per 1 cm^2). After that the liquid above the sediment was centrifuged at 180,000 g twice in two hours. The liquid above the sediment (centrifugate) was tested for the presence of an inhibitor capable of blocking the production and the effect of interferon. This was accomplished by using chicken fibroblasts grown within 5-6 days in 100 milliliter Pavitskaya's matrasses. All of the matrasses were divided into 6 groups: first group -- introduced 5 ml of centrifugate and 3 ml Chikungunya virus (with a titer of 10^6 TCD_{50}); second group -- 3 ml of Chikungunya virus; third group -- 5 ml of centrifugate; fourth group -- 5 ml of the centrifugate, 4 ml of interferon (with a titer of 1:128) and 10^3 TCD_{50} of Western equine encephalomyelitis virus; the fifth group -- 4 ml of interferon and 10^3 TCD_{50} of Western equine encephalomyelitis virus; the sixth group -- 10^3 TCD_{50} of Western equine encephalomyelitis virus. In a number of experiments, 10^2 TCD_{50} of vaccinia virus was introduced into the matrasses of the fourth-sixth groups instead of Western equine encephalomyelitis virus.

The matrasses of the first three groups with the tissue culture permitted to judge the ability of the inhibitor to block the production of interferon while the matrasses of the fourth-sixth groups served as a system for showing the ability of the inhibitor to inhibit the effect of interferon.

The volume of the liquid in all matrasses was brought to 15 ml with medium No. 199. All of the ingredients were added on the day when the centrifugate was obtained, and the indicator Western equine encephalomyelitis virus was introduced 24 hours later. The centrifugate was checked for the presence of residual virus which was not found in any of the experiments. A centrifugate of uninfected chicken fibroblasts treated in an analogous way was used as a control.

All of the matrasses were incubated at 37° in a thermostatically-controlled chamber until there appeared a degeneration of the cells of the chicken fibroblasts cultures infected by the virus alone. Then the contents of the matrasses of the first-third groups were treated with hydrochloric acid to pH 2.4 and 48 hours later to 7.4 and the content of interferon was determined. The matrasses with the cultures of the fourth-sixth groups were repeatedly frozen and thawed, after which the content of virus in them was determined by titration on chicken fibroblasts.

Results and Discussion

The results of the first series of experiments are given in Table 1 which shows that the centrifugate had a blocking effect on the production of interferon. The latter was found in a titer of 1:16 only in the cultures infected with the Chikungunya virus alone. It was completely absent in the cultures into which the virus and the centrifugate were introduced.

Table 1

Blocking of the Production of Interferon by Vaccinia Virus

Culture Group	Preparation added to cells	Titer of Interferon
1st	Centrifugate of infected cells	0
2nd	Chikungunya virus	1:16
3rd	Centrifugate and Chikungunya virus	0
Control	Centrifugate of normal cells	0

Along with this we studied the blocking effect of the inhibitor on the activity of interferon. However, we did not succeed in establishing the blocking effect on interferon when we used the virus of Western equine encephalomyelitis as a test virus. In the presence of the centrifugate, interferon completely inhibited the reproduction of Western equine ence-

phalomyelitis virus which is known to be highly sensitive to interferon. It was possible to assume that even if only a part of the interferon's activity was inhibited, the effect of the inhibitor would not show because of a high sensitivity of the test virus to interferon. This could be established either by using the limit dilutions of interferon or by using viruses which show a moderate sensitivity to the inhibiting effect of interferon. For this purpose we selected a strain of vaccinia virus obtained by the clone production method.

The results of the second series of experiments are given in Table 2. It can be seen that the blocking effect on the activity of interferon was detected only when vaccinia virus was used as a test virus. When the centrifugate was added to the virus-interferon mixture, it was noted that the reproduction of the virus was 100 times greater than in the cultures inoculated only with the virus and interferon. However, it should be noted that the blocking effect of the inhibitor in these experiments was only a partial one. The virus titer, in spite of the presence of the inhibitor in the medium, was 10 times lower than in the cultures infected with vaccinia virus alone. Consequently, the inhibitor forming in the cells of chicken fibroblasts under the effect of the vaccinia virus used is capable of blocking not only the production but also the effect of interferon.

In our further experiments we studied the effect of the introduction time of the inhibitor on the production of interferon when a Chikungunya virus was used as interferonogen. It was found that the blocking effect of the inhibitor on the formation of interferon was apparent only in those cases when the centrifugate was introduced into the culture no later than six hours after infecting the chicken fibroblasts with the interferonogen virus. A complete inhibition of the production of interferon was observed in cultures into which the virus and the centrifugate were introduced in succession with an interval of not more than one hour. When the intervals were 1-6 hours, the production of interferon gradually increased and was at a control level when the interval was more than six hours (Table 3).

As it has been mentioned before, a number of researchers were able to demonstrate the blocking effect of certain viruses on the formation and activity of interferon[3-6]. However, the authors of the above-mentioned works did not attempt to expose the inhibitor which, apparently, was stimulated by these viruses in the cells. Yu. Z. Gendon[3], who attempted to find the inhibitor blocking the formation of interferon, was not

Table 2

Blocking of the Effect of Interferon with a Nonviral Inhibitor

Preparation added to cells	Virus titer in TCD_{50}/ml
Centrifugate, vaccinia virus and interferon	10^4
Vaccinia virus and interferon	10^2
Vaccinia virus	10^5
Centrifugate, Western equine encephalomyelitis virus and interferon	0
Western equine encephalomyelitis virus and interferon	0
Western equine encephalomyelitis virus	10^7

Table 3

The effect of the interval between the introduction of the virus and the inhibitor on the formation of interferon

Preparation added to cells	Interval between the introduction of virus and centrifugate (in hours)	Interferon titer
Centrifugate and Chikungunya virus	0	0
" "	1	0
" "	3	1:4
" "	6	1:16
" "	8	1:32
" "	24	1:32
Chikungunya virus		1:32

successful. In our experiments which were based on a modified Yu. A. Gendon method, such an inhibitor was found, which was, apparently, connected with the fact that we used different viruses. It is interesting to note that the inhibiting effect of the centrifugate was expressed only during the first six hours after the introduction of the interferonogen. The results indicate that the obtainod inhibitor shows its effect

during an early stage in the production cycle of interferon synthesis. Further studies are required to pass a final judgment on the mechanism of the effect of the inhibitor. As for the inhibition of the activity of exogenic interferon by a centrifugate, it is not possible to say at the present time whether this effect is caused by the same inhibitor which blocks the formation of interferon. It is possible to assume that in the cells of chicken fibroblasts, the formation of two inhibitors different in nature is stimulated under the effect of the vaccinia virus used. However, this problem requires further research.

Conclusions

1. When monolayer cultures of chicken fibroblasts are infected with a used strain of vaccinia virus, the production of an inhibitor blocking the formation and effect of interferon is stimulated.

2. The blocking effect of the inhibitor becomes apparent when it is introduced not later than six hours after infecting the culture with interferonogen, which testifies to its effect on the early stages of the formation process of interferon.

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